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APPLICATION OF LAVENDER AND ROSEMARY ESSENTIAL OILS IMPROVEMENT OF THE MICROBIOLOGICAL QUALITY OF CHICKEN QUARTERS

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ABSTRACT

The aim of the present work was monitoring of chicken quarters microbiological indicators after treatment by ethylenediaminetetraacetate (EDTA), lavender (*Lavandula angustifolia* L.) and rosemary (*Rosmarinus officinalis* L.) essential oil, stored under vacuum packaging, at 4 ±0.5°C for a period of 16 days. The following treatments of chicken quarters were used: Air-packaging control samples, control vacuum-packaging samples, vacuum-packaging with EDTA solution 1.50% w/w, control samples, vacuum-packaging with *Lavandula angustifolia* essential oil at concentrations 0.2% v/w and vacuum-packaging with *Rosmarinus officinalis* essential oil at concentration 0.2% v/w. The quality assessment of all samples was established by microbiological analysis. Sampling was carried out after certain time intervals: 0, 4, 8, 12 and 16 days. Chicken quarters were stored under vacuum packaging, at 4 ±0.5°C during experiment. Microbiological analyses were conducted by using standard microbiological methods. Anaerobic plate count were determined using Plate Count Agar, after incubation for 2 days at 35°C under anaerobic condition. *Pseudomonas* spp. were determined on Pseudomonas Isolation agar after incubation at 48 h at 25°C. For lactic acid bacteria were inoculated into Rogosa and Sharpe agar after incubation 48-78 h at 37°C in an aerobic atmosphere supplemented with carbon dioxide (5% CO_2). For members of the family *Enterobacteriaceae* violet red bile glucose agar were used and samples were incubated at 37°C for 24 h. The initial APC value of chicken quarter was 3.00 log CFU.g^{-1} on 0 day. The number of anaerobic plate count ranged from 3.00 log CFU.g^{-1} in all tested group on 0 day to 6.11 log CFU.g^{-1} on 16 day in control group stored in air condition. The initial LAC value of chicken quarter was 3.00 log CFU.g^{-1} on 0 day. The number of lactic acid bacteria ranged from 3.00 log CFU.g^{-1} in all tested group on 0 day to 3.58 log CFU.g^{-1} on 16 day in control group stored in air condition. The initial *Enterobacteriacea* genera value of chicken quarter was 2.00 log CFU.g^{-1} on 0 day. Presences of these bacteria were found on all groups at 16 days. The results of this present study suggest the possibility of application the *Lavandula angustifolia* and *Rosmarinus officinalis* essential oil *as* natural food preservatives and potential sources of antimicrobial ingredients for food industry.

Keywords: chicken quarters; microorganisms; lavender and rosemary essential oils; vacuum; EDTA

INTRODUCTION

Chicken meat has many desirable nutritional characteristics such as a low lipid content and relatively high concentration of polyunsaturated fatty acids **(Bourre, 2005)**. Fresh meat products are usually marketed at refrigerated temperatures (2 – 5 °C). Spoilage of raw meat may occur in two ways during refrigeration: microbial growth and oxidative rancidity **(Sebranek et al., 2005; Zeleňáková et al., 2010)**. Spoilage of fresh poultry meat is an economic burden to the producer and it leads to the development of methods to prolong the shelf-life and overall safety/quality which is the major problem faced by poultry processing industry **(Petrou et al., 2012)**.

Furthermore, meats might get contaminated with microorganisms while butchering or during the manufacturing process, though the tissues of healthy animals would be sterile at the time of slaughter. These microorganisms bring about undesirable quality changes in meats, especially with respect to lactic acid bacteria, a major bacterial group associated with meat spoilage. When large numbers of microorganisms are present in raw meat, there will be changes such that it becomes unappealing and unsuitable for human consumption **(Gram et al., 2002; Doulgeraki et al., 2012)**.

Plants and plants products have been claimed to have health-promoting effects, which may be related to the antioxidant activity *in vivo* **(Ivanišová et al., 2013; Ivanišová et al., 2015 a,b)**.

At present, meat industry uses chemical additives in several meat processes to prevent the growth of food-borne pathogens and extend the shelf life of refrigerated storage. Since concern over the safety of chemical additives has

arisen in recent years, consumers increasingly demand the use of natural products as alternative preservatives in foods **(Govaris et al., 2010)**. Herbs and spices, which are important part of the human diet, have been used for thousands of years in traditional medicine and to enhance the flavor, color and aroma of foods. In addition to boosting flavor, herbs and spices are also known for their preservative **(Neilsen and Rios, 2000)**, antioxidative **(Shobana and Naidu, 2000)**, and antimicrobial roles.

The plants of *Lamiaceae* family are a rich source of polyphenols and hence could possess strong antioxidant properties **(Gulluce et al., 2007)**. *Lavandula latifolia*, known as Spike lavender or Portuguese lavender, is a flowering plant in the family *Lamiaceae*, native to the western Mediterranean region, from central Portugal to northern Italy (Liguria) through Spain and southern France. *L. latifolia* is a strongly aromatic shrub growing to 30 – 80 cm tall. The leaves are evergreen, 3 – 6 cm long and 5 – 8 mm broad. The flowers are pale lilac, produced on spikes 2 – 5 cm long at the top of slender, leafless stems 20 – 50 cm long. The flowers and leaves of this plant are used as an herbal medicine, in the form of an herbal tea. Lavender essential oil contains the most popular aromatic herbal compounds and is widely used in food, perfume and pharmaceutical industries **(Kim and Lee, 2002)**. Lavender essential oil has a soothing and calming effect on the nerves, relieving tension, depression, panic, hysteria and nervous exhaustion in general and is effective for headaches, migraines and insomnia. It is also very beneficial for problems such as bronchitis, asthma, colds, laryngitis, halitosis, throat infections and whooping cough. Many pharmacological benefits such as anticonvulsant, anxiolytic effect, antioxidant and anticholinesterases properties, anti-bacterial, antioxidant, anti-inflammatory, antimicrobial activity, antifungal activity, have been associated with this essential oil. Therefore, many researchers focus on analyzing Lavender essential oil. It is shown that the main chemical composition of Lavender essential oil depends on genotype, environment, processing and extraction methods **(Jalali-Heravi et al., 2015)**.

Rosmarinus officinalis L. (rosemary) is an aromatic plant belonging to *Lamiaceae* family **(Begum et al., 2013)**. Rosemary has been used for thousands of years for both culinary and medicinal proposes, due to its aromatic properties and health benefits. The biological activities of this plant are mainly related to the phenolic and the volatile constituents **(Babovic et al., 2010; Teixeira et al., 2013; Arranz et al., 2015)** such as carnosol, carnosic acid and rosmarinic acid present in the extract of rosemary and a-pinene, bornyl acetate, camphor and eucalyptol present in the essential oil of this plant **(Babovic et al., 2010; Teixeira et al., 2013; Arranz et al., 2015)**. Minor components may have a potential influence on the biological activity due to the possibility of synergistic effect among their components **(Hussain et al., 2010)**. *R. officinalis* L. can be used fresh, dried or as tea infusion.

The essential oil and the extract of rosemary can be obtained for application in food packaging, aromatherapy and medicine treatment **(Peter, 2004; Szumny et al., 2010; Amaral et al., 2013; Barreto et al., 2014)**. Rosemary is used for cooking as flavoring, in the preservation of foods, cosmetics **(Peter, 2004; Sasikumar, 2012)** or in folk medicine for anti-inflammatory, diuretic and antimicrobial applications **(Teixeira et al., 2013; Arranz et al., 2015)**. Currently, rosemary has been widely investigated as food additive. This aromatic plant can be added directly to food or incorporated in food packaging, performing as antimicrobial and antioxidant agent **(Liptajová et al., 2010; Ribeiro-Santos et al., 2015)**.

The aim of this study was to investigate the effects of lavender and rosemary essential oils, ethylenediaminetetraacetate in combination with vacuum packaging, on the microbiological indicators of chicken quarters.

MATERIAL AND METHODOLOGY

Preparation of samples

To evaluate the antimicrobial activity of essential oils the chicken quarters with skin of each experimental group was taken. The chicken quarters fresh samples were prepared as follow: for air-packaging (AC, control samples) chicken quarters fresh meat was packaged to polyethylene bags and stored aerobically in refrigerator; for vacuum-packaged (VPC, control samples) chicken quarters fresh meat was packaged to polyethylene bags and stored anaerobically in vacuum and in refrigerator; for vacuum-packed samples with EDTA solution 1.5% w/w (VPEC, control samples) chicken quarters fresh meat was treated with EDTA for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum and in refrigerator; for vacuum-packed samples with *Lavandula angustifolia* L. 0.20% v/w (VP+LAO) chicken quarters fresh meat was treated with lavender oil for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum and in refrigerator; for vacuum-packed samples with *Rosmarinus officinalis* L. 0.20 % v/w, (VP+ROO) chicken quarter fresh meat was treated with rosemary oil for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum and in refrigerator (4 ±0.5°C). For sample packaging a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic) was used and each sample were packed immediately after treatment. A stock solution of 500 mM concentration of EDTA was prepared by diluting 186.15g in 1 L distilled water (EDTA, $C_{10}H_{14}N_2O_8.Na_2.2H_2O$), 99.5% purity, analytical grade, (Invitrogen, USA). A final concentration of 50 mM EDTA solution was prepared from the stock solution. The pH of the solution was adjusted to 8.0 with the addition of the appropriate quantity of NaOH solution. Lavender and rosemary essential oil (Hanus, Nitra, Slovakia) was added to coat chicken quarter surface (both sides) of each sample using a micropipette. Final concentration of 0.2% v/w of EO was used for treatment.

Microbiological analysis

Approximately 10 g (10 cm^2) of the chicken quarter was sampled using sterile scalpels and forceps, immediately transferred into a sterile stomacher bag, containing 90 mL of 0.1% peptone water (pH 7.0), and homogenized for 60 s in a Stomacher at room temperature. Sampling was carried out after certain time intervals: 0, 4, 8, 12 and 16 days. Chicken quarters were stored under vacuum packaging, at

4 ±0.5°C during experiment. Microbiological analyses were conducted by using standard microbiological methods. Anaerobic plate count (APC) were determined using Plate Count Agar (PCA, Oxoid, UK), after incubation for 2 days at 35°C under anaerobic condition. For *Pseudomonas* spp. enumerations, 0.1 mL from 1:10 prepared serial dilutions (0.1% physiological solution) of chicken homogenates was spread onto the surface of solid media. *Pseudomonas* were determined on Pseudomonas Isolation agar (PIA, Oxoid, UK) after incubation at 48 h at 25°C. For lactic acid bacteria enumeration, a 1.0 mL sample were inoculated into Rogosa and Sharpe agar (MRS, Oxoid, UK) after incubation 48 – 78 h at 37°C in an aerobic atmosphere supplemented with carbon dioxide (5% CO_2). For members of the family *Enterobacteriaceae*, a 1.0 mL sample was inoculated into 10 mL of molten (45°C) violet red bile glucose agar (VRBL, Oxoid, UK). After setting, a 10 mL overlay of molten medium was added and samples incubated at 37°C for 24 h. The large colonies with purple haloes were counted. All plates were examined for typical colony types and morphology characteristics associated with each medium applied for incubation.

RESULTS AND DISCUSSION

The main driving force for the growth of worldwide food industry is the scope and range of food preservation and shelf life extension technology **(Sadaka et al., 2013)**. Active packaging is gaining increasing attention from researchers and the industry due to its potential to provide quality and safety benefits. Active packaging is a type of packaging that changes its conditions as a way to extend life or enhance safety or sensory properties while maintaining food quality **(Martucci et al., 2015)**. In view of the health concerns expressed by consumers and current environmental problems, research is now focusing on the development of sustainable packaging materials based on annually renewable natural biopolymers such as polysaccharides and proteins **(Gomez-Estaca et al., 2010)**.

The consumer's desire for natural ingredients and for chemical preservative-free foods has increased the popularity of natural antimicrobial agents **(Sadaka et al., 2013)**. In this framework, the addition of essential oils to biopolymer films as natural bacteriostatics could be an interesting election. Essential oils have well-recognized properties, such as antimicrobial **(Gende et al., 2010; Teixeira et al., 2013 a,b)**, antibacterial **(Min and Oh, 2009; Teixeira et al., 2013 a,b)** and antioxidant properties **(Burt, 2004; Danh et al., 2012; Kacániová et al., 2012)**.

Anaerobic plate count (APC) values for the tested groups of chicken quarter are showed in Figure 1. The initial APC value of chicken quarter was 3.00 log $CFU.g^{-1}$ on 0 day. The number of anaerobic plate count ranged from 3.00 log $CFU.g^{-1}$ in all tested group on 0 day to 6.11 log $CFU.g^{-1}$ on 16 day in control group stored in air condition. In control group stored in air condition the number of APC ranged from 3.00 log $CFU.g^{-1}$ on 0 day to 6.11 log $CFU.g^{-1}$ on 16 day. In control group stored under vacuum packaging ranged from 3.00 log $CFU.g^{-1}$ on 0 day to 6.05 log $CFU.g^{-1}$ on 16 day. In control group stored under vacuum packaging and EDTA treatment APC ranged from 3.00 log $CFU.g^{-1}$ on 0 day to 5.75 log $CFU.g^{-1}$ on 16 day. In the group with lavender essential oil treatment number of APC ranged from 3.00 log $CFU.g^{-1}$ on 0 day to 5.21 log $CFU.g^{-1}$ on 16 day and in group with with rosemary essential oil treatment ranged from 3.00 log $CFU.g^{-1}$ on 0 day to 4.98 log $CFU.g^{-1}$ on 16 day.

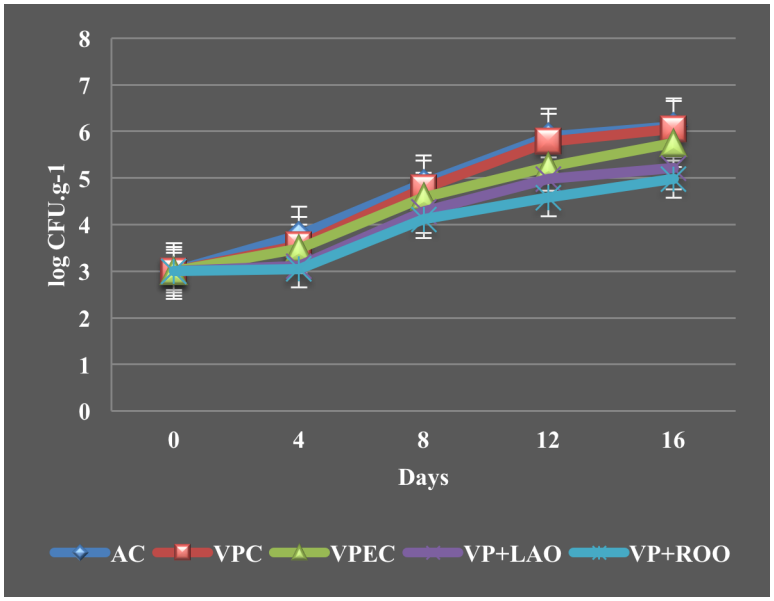

Figure 1 Changes (log $CFU.g^{-1}$) in population of anaerobic plate count in chicken quarter stored in air (AC); stored under vacuum (VPC); stored under vacuum packaging with EDTA (VPEC); stored under vacuum packaging with *Lavandula angustifolia* L. 0.20% v/w (VP+LAO); stored under vacuum packaging with *Rosmarinus officinalis* L. 0.20 % v/w, (VP+ROO).

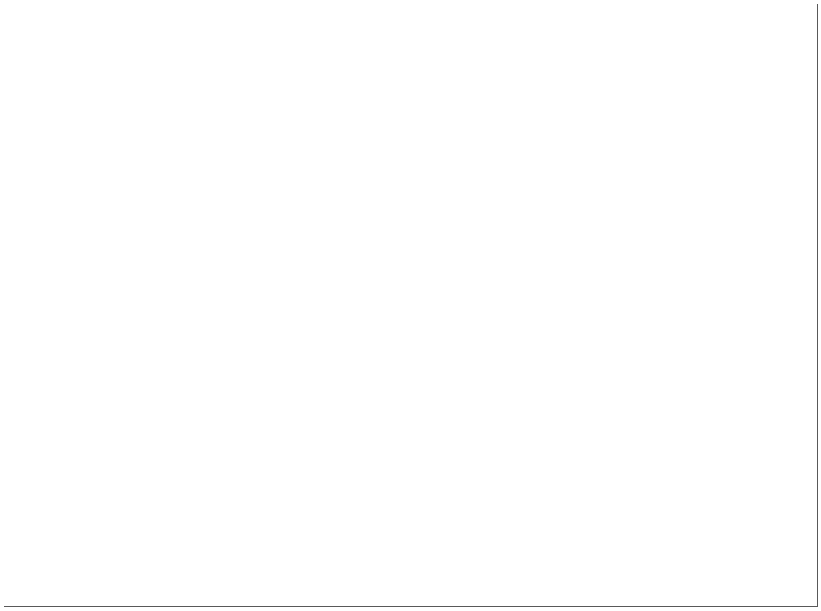

Figure 2 Changes (log CFU.g^{-1}) of lactic acid bacteria in chicken quarter stored in air (AC); stored under vacuum (VPC); stored under vacuum packaging with EDTA (VPEC); stored under vacuum packaging with *Lavandula angustifolia* L. 0.20% v/w (VP+LAO); stored under vacuum packaging with *Rosmarinus officinalis* L. 0.20 % v/w, (VP+ROO).

Lactic acid bacteria (LAB) values for the tested groups of chicken quarter are showed in Figure 2. The initial LAC value of chicken quarter was 2.00 log CFU.g^{-1} on 0 day. The number of lactic acid bacteria ranged from 2.00 log CFU.g^{-1} in all tested group on 0 day to 3.58 log CFU.g^{-1} on 16 day in control group stored in air condition. The number of lactic acid bacteria ranged from 2.00 log CFU.g^{-1} in all tested group on 0 day to 3.58 log CFU.g^{-1} on 16 day in control group stored in air condition. In control group stored in air condition the number of LAB ranged from 2.00 log CFU.g^{-1} on 0 day to 3.58 log CFU.g^{-1} on 16 day. In control group stored under vacuum packaging ranged from 2.00 log CFU.g^{-1} on 0 day to 3.24 log CFU.g^{-1} on 16 day. In control group stored under vacuum packaging and EDTA treatment LAB ranged from 2.00 log CFU.g^{-1} on 0 day to 3.12 log CFU.g^{-1} on 16 day. In the group with lavender essential oil treatment number of LAC ranged from 2.00 log CFU.g^{-1} on 0 day to 2.89 log CFU.g^{-1} on 16 day and in group with with rosemary essential oil treatment ranged from 2.00 log CFU.g^{-1} on 0 day to 2.85 log CFU.g^{-1} on 16 day.

The addition of rosemary or thyme EO to fine paste meat products has been effective against aerobic bacteria and LAB **(Viuda-Martos et al., 2010)**.

The antimicrobial effects of different spice extracts in raw chicken meat during storage for 15 days at 4 °C were studied in **Radha Krishan et al. (2014)** work. Raw chicken meat was treated with BHT (positive control), *Syzygium aromaticum*, *Cinnmomum cassia*, *Origanum vulgare*, and *Brassica nigra* extracts and the different combinations as well as the results were compared to raw chicken meat without any additive (negative control). The antimicrobial activities of spice extracts were determined.

The total viable counts, Lactic Acid Bacteria counts, *Enterobacteriaceae* counts, *Pseudomonas* spp. cunts were determined at a gap of 3 days interval for a period of 15 days. The bacterial counts of groups with spice samples were lower than control samples during storage.

EOs have been shown to possess antibacterial and anti-fungal activities against several microorganisms associated with meat, including gram-negative and gram-positive bacteria **(Karabagias et al., 2011)**. Many recent studies have been conducted to examine the effects of EOs obtained from sour-ces such as oregano, rosemary, thyme, sage, basil, turmeric, coriander, ginger, garlic, nutmeg, clove, mace, savory, and fennel, when used alone or in combination with other EOs and/or preservation methods, in order to improve the sensory qualities and extend the shelf life of meat and meat products **(Goulas and Kontominas, 2007)**. Additionally, extracted EOs and oleoresins are preferred over crude spices in the meat in-dustry due to their better stability during storage, microbial safety, high concentration of flavor components, reduced storage space, ease of handling, no seasonal variation, and standardization **(Jayasena and Jo, 2013)**.

Numerous experimental applications of EOs as antimicrobial agents in meat and meat products are summarized. **Skandamis et al., (2002)** reported that EOs from clove, oregano, rosemary, thyme, and sage have high inhibitory activity, particularly against gram-positive bacteria, rather than gram-negative bacteria **(Marino et al., 2001)**.

Chicken treated with thyme EO has been reported to show remarkable inhibition of lactic acid bacteria (LAB) growth until the end of storage. This agrees well with the findings of **Gutierrez et al., (2009)** on the inhibitory action of these oils against *E. coli* and *Salmonella* spp. in food as well as in *in vitro* models.

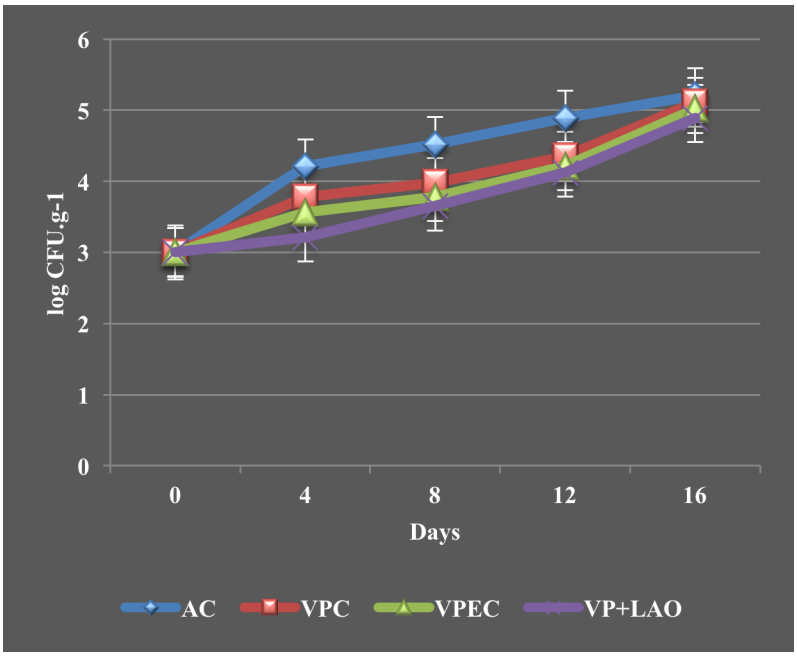

Figure 3 Changes (log CFU.g^{-1}) in population of *Enterobacteriaceae* genera in chicken quarter stored in air (AC); stored under vacuum (VPC); stored under vacuum packaging with EDTA (VPEC); stored under vacuum packaging with *Lavandula angustifolia* L. 0.20% v/w (VP+LAO); stored under vacuum packaging with *Rosmarinus officinalis* L. 0.20% v/w, (VP+ROO).

Clove, cinnamon, pimento, and rosemary EOs effectively inhibited the growth of meat spoilage bacteria. Further, cumin, garlic, oregano, and black pepper EOs considerably inhibited the growth of meat spoilage organisms. Similar results had been reported previously by **Aureli et al., (1992)**, who examined the inhibitory effect of EOs against various pathogens and spoilage bacteria.

Enterobacteriaceae genera values for the tested groups of chicken quarter are showed in Figure 3. The initial *Enterobacteriacea* genera value of chicken quarter was 2.00 log CFU.g^{-1} on 0 day. Presences of these bacteria were found on all groups at 16 day.

The presence of *Pseudomonas* spp. bacteria in this study were not found in all tested groups.

Main spoilage bacteria including *Pseudomonas, Acinetobacter, B. thermosphacta, Moraxella, Enterobacter*, *Lactobacillus* spp., *Leuconostoc* spp., *Proteus* spp. etc, yeast and mold decompose meat and meat products and develop unpleasant quality characteristics **(Fratianni et al., 2010; Lucera et al., 2012)** when they grow in large number in these perishable products.

The mechanism of action for the antimicrobial activity of spice and plants extracts is not fully understood, however, membrane disruption by phenolics and metal chelation by flavonoids are considered to inhibit the growth of microorganisms. Some researchers have reported that phenolic compounds from different plant sources could inhibit various food-borne pathogens, and the total phenolic content have been highly correlated with antibacterial activity **(Shan et al., 2007)**. The antimicrobial activities of phenolic compounds may involve multiple modes of action. For example, phenolic compounds can degrade the cell wall, disrupt the cytoplasmic membrane, cause leakage of cellular components, change fatty acid and phospholipid constituents, influence the synthesis of DNA and RNA and destroy protein translocation **(Shan et al., 2007)**.

CONCLUSION

Lavender and rosemary essential oils exhibited good antimicrobial properties against anaerobic bacteria, lactic acid bacteria and *Enterobacteriacea* genera in 0.2% concentration. Meat is highly subject to microbial deterioration, which ultimately leads to safety and quality issues if the meat is not properly handled and preserved. Several plant-derived EOs can be effectively used in meat as natural alternatives to synthetic food additives, particularly as effective antimicrobial agents.

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